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TITLE: The Role of NFIB in Prostate Cancer Progression

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CONTRACTING ORGANIZATION: Vanderbilt University
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14. ABSTRACT
Nuclear factor I/B (NFIB) is a co-factor of androgen receptor (AR) and FOXA1. Previous studies in the laboratory have suggested a role for NFIB in controlling neuroendocrine differentiation (loss of AR expression, gain of neuroendocrine markers like synaptophoysin) during prostate cancer progression. Knockdown of NFIB expression in LNCaP and C4-2B prostate cancer cell lines results in increased AR message and protein levels, but synaptophysin levels were not affected. 22RV1 cells, which are not reported to undergo neuroendocrine differentiation, expressed synaptophysin and AR at an increased level in response to NFIB over-expression. 22RV1-NFIB cells were also more proliferative than their 22RV1-vector controls in both serum-containing and serum-free media. These studies are defining a role for NFIB regulation of AR and synaptophysin in prostate cancer *in vitro* and suggest a role for NFIB during prostate cancer progression *in vivo.*

15. SUBJECT TERMS
Prostate cancer, therapy acquired neuroendocrine prostate cancer, neuroendocrine prostate cancer, androgen receptor, NFIB

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1. INTRODUCTION:

Castrate resistant prostate cancer (CRPCa) maintains androgen receptor (AR) signaling but is no longer responsive to androgen deprivation therapy (ADT). This resistance is driven by multiple factors, including the expression of AR splice variants (AR-V) and the progression of neuroendocrine differentiation (NED) to therapy acquired neuroendocrine prostate cancer (NEPCa). However, new therapies continue to target the AR ligand binding domain, which is missing in AR-V, or altogether absent in therapy (t) induced NEPCa (tNEPCa). Our strategy, therefore, has focused on targeting AR via its co-factors, such as FOXA1 and the Nuclear Factor I family members (NFIA, NFIB, NFIC, and NFIX). We postulate that these transcription factors will have functions dependent on AR interaction, as well as independent functions. Our work and the work of others have demonstrated that neuroendocrine (NE) tumors can support castrate-resistant growth of prostate adenocarcinomas and that NED occurs frequently in CRPCa. Based on preliminary data, we believe that the transcription factor NFIB is a likely candidate for controlling AR expression and NED. It is possible, therefore, that patients who maintain high levels of NFIB will be more likely to undergo NED and fail ADT. The purpose of these studies has been two-fold. First, by analyzing clinical samples, we seek to determine whether NFIB expression in human PCa samples can predict biochemical recurrence. Secondly, by exploring the role of NFIB in driving NED in PCa cell lines *in vitro* and *in vivo*, we will define a mechanistic role for NFIB in driving NED. Successful completion of these studies may provide a novel biomarker of aggressive PCa, as well as define a role for NFIB in therapeutic failure.

2. KEYWORDS:

Prostate cancer, therapy acquired neuroendocrine prostate cancer, neuroendocrine prostate cancer, androgen receptor, NFIB

3. ACCOMPLISHMENTS:

- **What were the major goals of the project?**

The major goals of this project were to further my training in prostate cancer research, determine whether NFIB expression was a predictor of early biochemical recurrence in prostate cancer patients, as well as explore how NFIB could drive NED and support castrate resistant growth through neuroendocrine secretions. <u>These goals were to be achieved through the following approved statement of work:</u>

Training-Specific Tasks:

Major Task 1: Training and educational development in prostate cancer research *(only applicable to training award mechanisms)*	**Month**	**VUMC**
Subtask 1: Weekly meetings with mentors	1-24	Dr. Grabowska
Subtask 2: Present research at biweekly lab meetings and VUMC Prostate Cancer Center (VPCC) department group meetings	1-24	Dr. Grabowska
Subtask 3: Attend Translational Seminar Bridge Series and Urologic Oncology meetings	1-24	Dr. Grabowska
Subtask 4: Attend Society of Basic Urologic Research (SBUR), American Urologic Association (AUA), and IMPACT meetings.	1-24	Dr. Grabowska
Milestone(s) Achieved: Presentation of project data at a national meeting, submission of manuscripts, preparation of K99/R00 grant application on basis of studies	24	

Research-Specific Tasks:

Specific Aim 1: Examine if NFIB expression predicts aggressive disease by supporting failure of ADT via NED.		
Major Task 1: Determine whether NFIB correlates with aggressive disease, NED, and poor clinical outcome in human PCa patients.		
Subtask 1: Obtain exempt IRB approval (Months 0-3)	1-3	Dr. Grabowska
Subtask 2: Patient review by Dr. Clark and request appropriate de-identified slides from Pathology	3-9	Dr. Grabowska
Subtask 3: Perform immunohistochemistry for NFIB and have it scored (Dr. Justin Cates, Pathology)	10-12	Dr. Grabowska
Subtask 4: With assistance from Dr. Clark, co-mentor, determine whether NFIB expression predicts NED and failure of therapy.	12-13	Dr. Grabowska
Major Task 2: Generate and characterize non-targeting shNT(control), shNFIB, and NFIB over-expressing cell lines		
Subtask 1: Transduce cell lines with non-targeting (shNT), shNFIB, PCH (vector) or NFIB constructs Cell lines used: LNCaP, 22RV1, PC-3, and DU145 [AATC]; C4-2B [no longer commercially available, but established in laboratory]	1-6	Dr. Grabowska
Subtask 2: Examine cell lines generated in Aim 1b (subtask 1) for changes in AR, ARvs, NFIB, and markers of NED (SYN, CHGA, NSE) Cell lines used: LNCaP, 22RV1, PC-3, and DU145 [AATC]; C4-2B [no longer commercially available, but established in laboratory]	6-12	Dr. Grabowska
Subtask 3: Determine whether LNCaP and C4-2B cells generated in Aim 1b can no longer undergo NED in the absence of NFIB Cell lines used: LNCaP [AATC] and C4-2B [no longer commercially available, but established in laboratory]	6-9	Dr. Grabowska
Subtask 4: Determine whether 22RV1 cells generated in Aim 1b gain the ability to undergo NED with NFIB over-expression as compared to EV cells. Cell lines used: 22RV1 [AATC]	7-10	Dr. Grabowska
Subtask 5: If knockdown of NFIB in PC-3 or DU145 cells generated in Aim 1b restored AR expression, perform ChIP-seq to determine AR and NFIB binding sites/target genes	6-9	Dr. Grabowska
Subtask 6: Examine the consequence of NFIB loss in response to androgens (DHT) and anti-androgens (bicalutamide [Bic] and enzalutamide [Enz]) in cell lines generated in Aim 1b. Cell lines used: LNCaP, 22RV1, PC-3, and DU145 [AATC]; C4-2B [no	10-12	Dr. Grabowska

longer commercially available, but established in laboratory]		
Milestone(s) Achieved: Determination whether NFIB expression predicts aggressive PCa, supports NED in vitro	14	Dr. Grabowska
Specific Aim 2: Examine the role of NFIB during ADT response in vivo.		
Major Task 1: Perform xenograft experiments with the cell lines generated in Task 2		
Subtask 1: Generate twelve grafts per cell line established in Aim 1b. After three weeks, castrate half of the animals (proxy for ADT) for 2 weeks. Cell lines used: LNCaP, 22RV1, PC-3, and DU145 [AATC]; C4-2B [no longer commercially available, but established in laboratory] Mice: 12 mice per group, 5 cell lines, 2 groups [120 total]	12-15	Dr. Grabowska
Subtask 2: Stain grafts by immunohistochemistry (IHC) for NFIB, luminal markers (AR, CK8/18, PSA), basal markers (p63, CK14), and NED markers (SYN, CHGA, NSE)	14-17	Dr. Grabowska
Major Task 2: Generate and characterize NE-10 shNFIB and non-targeting shNT cell lines		
Subtask 1: Utilizing lentiviral transduction, generate shNFIB and shNT NE-10 cell lines. Cell lines: mouse neuroendocrine PCa, NE-10 [Matusik laboratory]	13-17	Dr. Grabowska
Subtask 2: Characterize the shNFIB and shNT NE-10 cell lines for global changes mediated by NFIB via RNA-seq Cell lines: mouse neuroendocrine PCa, NE-10 [Matusik laboratory]	18-20	Dr. Grabowska
Major Task 3: Establish dual xenografts with NE-10 and LNCaP cells		
Subtask 1: Athymic nude mice will host the following combinations of subcutaneous xenografts: NE-10 shNFIB + LNCaP shNT; NE-10 shNT + LNCaP shNT; NE-10 shNFIB + LNCaP shNFIB; NE-10 shNT + LNCaP shNFIB. Once tumors are established, animals will be castrated as a proxy for ADT Cell lines: LNCaP [AATC], mouse neuroendocrine PCa, NE-10 [Matusik laboratory] Mice: 12 mice per group, 4 groups, groups [48 mice total]	20-24	Dr. Grabowska
Subtask 2: Analyze tumors for tumor volume, mitotic index, invasion, and markers of NED (SYN, CHGA, NES)	22-24	Dr. Grabowska
Milestone(s) Achieved: Characterize the role of NFIB during NED in vivo, publication of manuscripts	24	

- **What was accomplished under these goals?**

THIS SECTION CONTAINS UNPUBLISHED DATA

__Training Tasks__

Major activity/task 1: Training and educational development in prostate cancer research

Objective: The objective of this first task is to expand my training and education in prostate cancer research through meetings with my mentors (Drs. Matusik and Clark), departmental meetings, and attending national conferences.

Results/achievements: During the reporting period, I met with Dr. Matusik (primary mentor) at least once a week and with Dr. Clark (clinical co-mentor) monthly. I attended the monthly Translational Bridge Seminar Series and the bi-monthly Urologic Oncology Conference. I also attended annual meeting of the SBUR in Dallas, TX. In addition to the outlined tasks, I was also invited and attended the American Association for Cancer Research (AACR) Translational Cancer Research for Basic Scientists Workshop.

START OF UNPUBLISHED DATA

__Research Tasks__

Specific Aim 1

Major activity/task 1: Determine whether NFIB correlates with aggressive disease, NED, and poor clinical outcome in human PCa patients.

Objective: The objective of this first task of the research tasks is to examine human prostate cancer samples and determine whether NFIB expression predicts poor clinical outcome and if NFIB is associated with NED.

Results/achievements: This aim is still in progress and will be completed after the termination of the DOD Postdoctoral Fellowship. Thus far, I have generated the data collection sheet (See Appendices) and have received Institutional Review Board (IRB) approval. I have also developed a database within the RedCap systems to store the data collection sheets behind a password protected firewall and without identifiable information. I have been slowly navigating the excellent Vanderbilt University Medical Center (VUMC) resources, such as the biostatistics clinic to determine the optimal study design and patient number, as well the Vanderbilt Institute for Clinical and Translational Research (VICTR) for funding. Our initial study design, which proposed to look at prostate cancer tumors (1 case/slide) proved cost and effort-prohibitive, and we have transitioned into generating a tissue microarray so that we can evaluate multiple patients per slide. By working with the Vanderbilt Biostatistics clinic, we have determined that the minimal requirement for the microarray will be 69 patients in each arm of a matched case-control study. This number of patients falls well within the resources of the Vanderbilt Urologic Outcomes Database.

Also, during the tissue microarray design stage, I worked with the VUMC Translational Pathology Shared Resource Core to obtain an estimate for the cost of generating the microarray, which I hoped to fund through the VCTR resource. However, because the prostate cancer samples of interest are heavily sought after and each sample must be reviewed by a pathologist, we have opted not to duplicate effort with other investigators at Vanderbilt. As such, we are working with Dr. Pietenpol, the Director of the Vanderbilt-Ingram Cancer Center, who is currently generating a tissue microarray that will satisfy the requirements for our study. The tissue microarray has been designed as a nested case control study with clinical data derived from the Urologic Outcomes database from which Dr. Clark and I were working. In the cohort, half of the patients have had a biochemical recurrence, while the other half has not. Patients were matched on multiple factors, including stage, grade, and surgical margin status. Each patient has two cores represented in the tissue microarray. We are currently waiting for the completion of a tissue microarray to finish our studies.

Major activity/task 2: Generate and characterize non-targeting shNT(control), shNFIB, and NFIB over-expressing cell lines.

Objective: The objective of this aim was to generate NFIB knockdown cell lines (LNCaP, C4-2B, PC3, and DU145) and determine the consequences of NFIB loss. For LNCaP and C4-2B cells, we hypothesized that loss of NIFB would prevent NED (loss of AR, gain of synaptophysin), alter the response to DHT and sensitivity to bicalutamide, an anti-androgen. Similarly, 22RV1 cells, which do not undergo NED in response to ADT, were used to determine if NFIB over-expression was sufficient to induce NED. PC-3 and DU145 cells are AR negative, and were used to determine if loss of NFIB was sufficient to re-express AR protein expression.

Results/achievements:

Cell line generation:

Five prostate cancer cell lines were used in the studies. LNCaP and its sub-line C4-2B both express AR and can undergo NED[1]. These cells were used to determine whether NFIB is required to undergo NED. Like C4-2B cells, 22RV1 cells express both AR and AR-V[2], and are castrate resistant, but they do not undergo NED in charcoal stripped serum conditions[1]. These cells were utilized to determine if NFIB alone is sufficient to induce NED. Finally, PC-3 and DU145 cells lack AR, are classified as androgen independent, and do not undergo NED in charcoal stripped serum[1]. These cells were used to determine whether ablation of NFIB was sufficient to induce re-expression of AR.

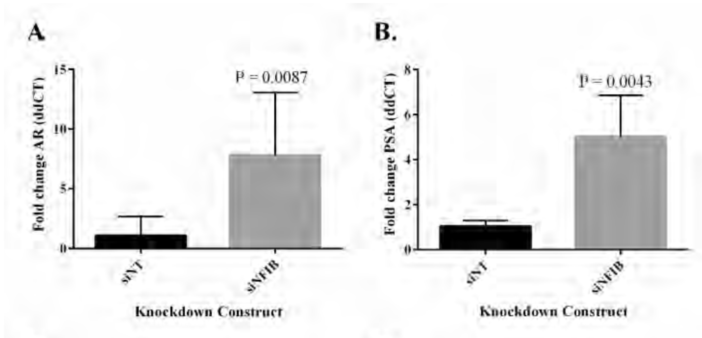

Figure 1: NFIB is not sufficient to prevent NED in LNCaP cells. Expression of *AR, PSA* (AR target gene), and neuronal markers (*SYP*, *ENO2*) in response to transient knockdown of NFIB under serum-free conditions. NT: non-targeting.

For these studies, a Myc tagged- NFIB over-expression construct (NFIB-Myc, RC501590) and short hairpin RNA constructs targeting NFIB (shNFIB-GFP, TG311191) were purchased from Origene (Rockville, MD). The following cell lines have been generated through transfections with lipofectamine 2000 and selection with media supplemented with antibiotic (puromycin for knockdown cell lines and neomycin for over-expressing cell line):

1. 22RV1: 22RV1-Vector, 22RV1-NFIB
2. LNCaP: LNCaP-shScramble (control), LNCaP-shNFIB #1, LNCaP-shNFIB #2, LNCaP-shNFIB #3, LNCaP-shNFIB #4
3. C4-2B: C4-2B-shScramble (control), C4-2B-shNFIB #1, C4-2B-shNFIB #2, C4-2B-shNFIB #3, C4-2B-shNFIB #4
4. PC3: PC3-shScramble (control), PC3-shNFIB #1, PC3-shNFIB #2, PC3-shNFIB #3, PC3-shNFIB #4
5. DU145: DU145-shScramble (control), DU145-shNFIB #1, DU145-shNFIB #2, DU145-shNFIB #3, DU145-shNFIB #4

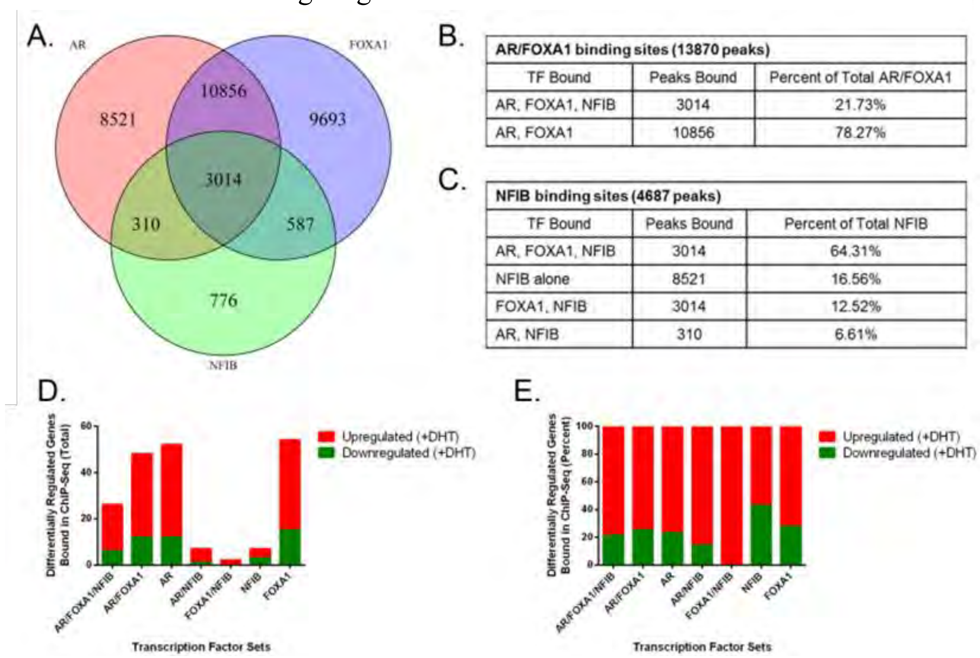

Figure 2: NFIB is frequently associated with AR and FOXA1 binding sites. A. Venn diagram of Chromatin immunoprecipitation then sequencing (ChIP-Seq) overlapping sites for AR, FOXA1, and NFIB. **B.** Breakdown of transcription factors associated with AR/FOXA1 peaks. **C.** Breakdown of transcription factors associated with NFIB peaks. **D.** Differentially regulated genes bound by transcription factor sets. **E.** Percentage of differentially regulated genes bound by transcription factor sets.

While cell lines were being established and characterized, preliminary studies were conducted using transient transfections of a previously published short-interfering RNA (siRNA) for NFIB [3].

<u>NFIB is not sufficient to prevent NED:</u>

LNCaP cells, which can undergo NED in response to serum withdrawal, were transiently transfected with non-targeting siRNA or NFIB targeting siRNA (siNT, siNFIB) and placed in phenol-red free, serum-free media for three days (Figure 1). Cells were then collected, RNA was extracted, and quantitative real time PCR (qRT-PCR) was performed for markers of NED. NED is characterized by the loss of AR and AR-target gene expression (prostate specific antigen [*PSA*] with a concomitant gain of neuronal marker expression (synaptophysin [*SYP*], neuronal specific enolase [*ENO2*]). We hypothesized that loss of NFIB would prevent LNCaP cells from undergoing NED, mainly cells would maintain expression of *AR* and lose expression of *SYP* and *ENO2*. Analysis of transiently transfected LNCaP cells revealed that while loss of NFIB did increase *AR* and *PSA* expression (Figure 1A, B), it was not sufficient to prevent expression of *SYP* or *ENO2*, suggesting that while NFIB can regulated the expression of AR and AR-target genes, its downregulation is not sufficient to prevent the expression of genes associated with NED.

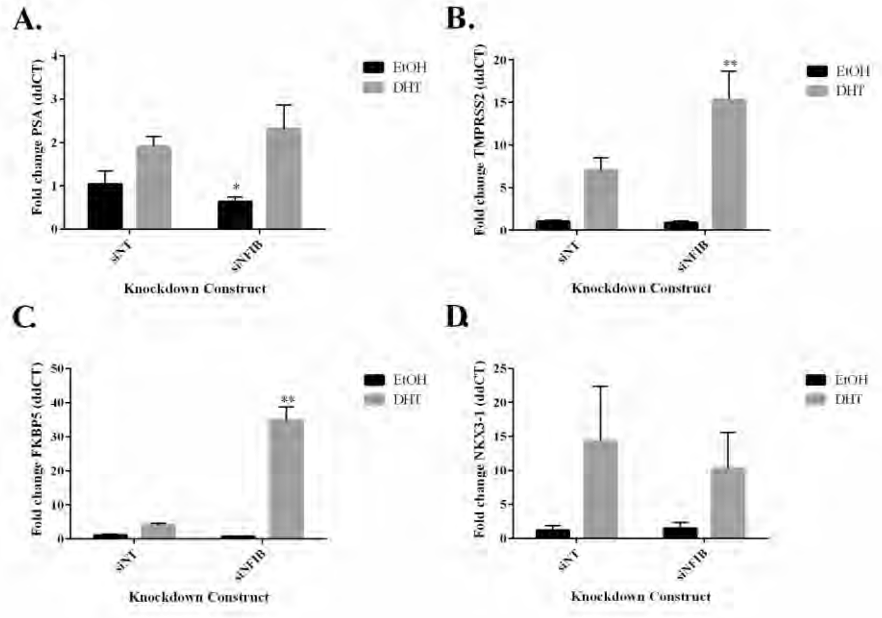

Figure 3: NFIB represses AR-target gene expression. Expression of *PSA* **(A)**, *TMPRSS2* **(B)**, *FKBP5* **(C)**, and *NKX3-1* **(D)** in response to DHT and and *NFIB* knockdown. NT: non-targeting. * P< 0.05, ** P < 0.01

<u>NFIB modulates AR and AR-target gene expression in response to DHT</u>

Transient transfection experiments in LNCaP cells also explored the role of NFIB in controlling AR/FOXA1/NFIB target genes. Our previous studies have demonstrated that while NFIB plays a largely repressive role in AR-target gene expression under serum-containing conditions[3]. More recent chromatin immunoprecipitation then DNA sequencing (ChIP-Seq) studies has explored how frequently NFIB binding sites are associated AR/FOXA1 binding sites (Figure 2A, B, C). I observed that AR/FOXA1/NFIB binding sites are largely associated with genes whose expression increases following DHT exposure (Figure 2D, E). However, when NFIB is knocked down, the gene expression of these genes (*TMPRSS2, FKBP5*) increases even further, suggesting that the role of NFIB in this complex may serve as a brake to limit transcription (Figure 3A, B, C, and D). Interestingly, NFIB can also repress *AR* and *AR-V7* message (Figure 4), suggesting that NFIB regulates AR expression under both serum-free and serum-containing conditions.

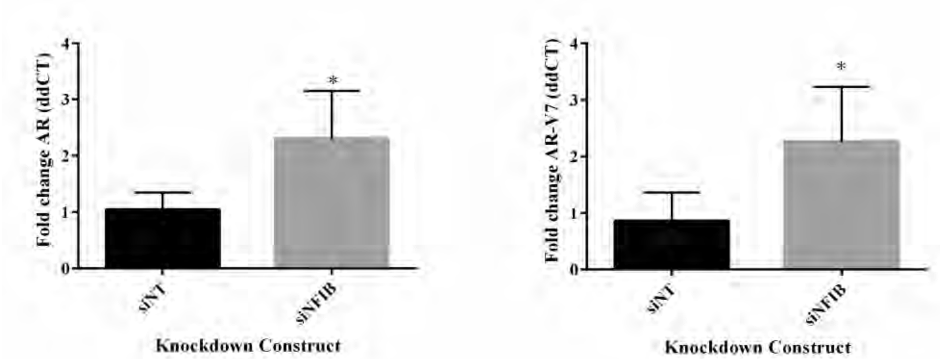

Figure 4: NFIB controls AR and AR-V7 expression. Transient knockdown of *NFIB* in LNCaP cells under serum containing conditions results in increase *AR* and *AR-V7* message. * P< 0.05

In preliminary studies utilizing the stable knockdown cell lines, this observation holds true, and knockdown of NFIB in LNCaP cells results in increased expression of AR (Figure 5A). However, the situation becomes more complex in the castrate resistant cell lines. In C4-2B cells, knockdown of NFIB has a variable response, with a loss of AR-V expression across all knockdown clones, but full length AR expression is variable (Figure 5A). In 22RV1 cells, which are castrate resistant but are not reported to undergo NED, over-expression of NFIB appears to increase full length AR and AR-V expression. Interestingly, AR expression increases under serum-free conditions, as does SYP expression in 22RV1 (Figure 5B). This observation suggests that NFIB over-expression in 22RV1 can induce both AR and SYP under serum-free conditions. This finding is quite unexpected, and subsequent studies are evaluating whether these changes in AR and SYP are occurring in the same or whether NFIB over-expression has generated a heterogeneous population. It should be noted that while knockdown of NFIB is sufficient to induce an increase in AR gene expression in PC-3 and DU145 cells it is not sufficient to re-express AR protein (data not shown), suggesting that the loss of AR expression in androgen independent cells is a multi-factorial process.

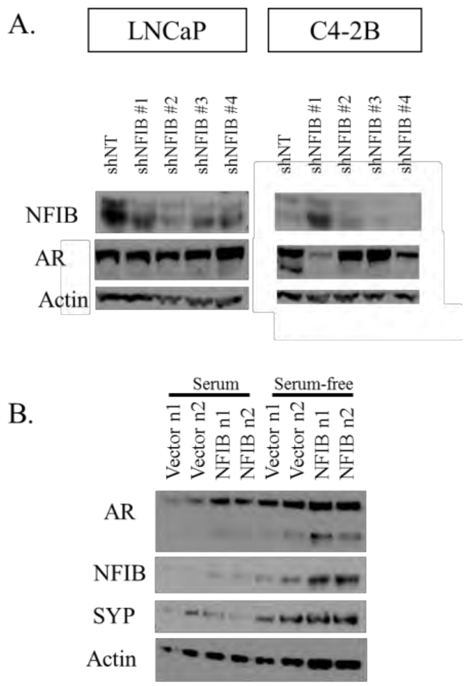

Figure 5: NFIB modulates AR expression. A. Western blot characterization of LNCaP and C4-2B NFIB-knockdown cells for AR expression. **B.** Western blot analysis of 22RV1 cells over-expressing NFIB in response to one week of serum-free media.

<u>NFIB over-expression drives increased androgen-independent proliferation:</u>

Proliferation studies utilizing our 22RV1-NFIB and vector control cells have demonstrated that 22RV1-NFIB cells are more proliferative than their vector control counterparts under serum-containing and serum-free conditions (Figure 6A, B). Notably, NFIB over-expression in cells resulted in a significant increase in growth rate in the presence of DHT (Figure 6C), likely due to the increased levels of AR. Expression of NFIB, however, did not sensitize the cells to anti-androgen (Bicalutamide, Figure 6D).

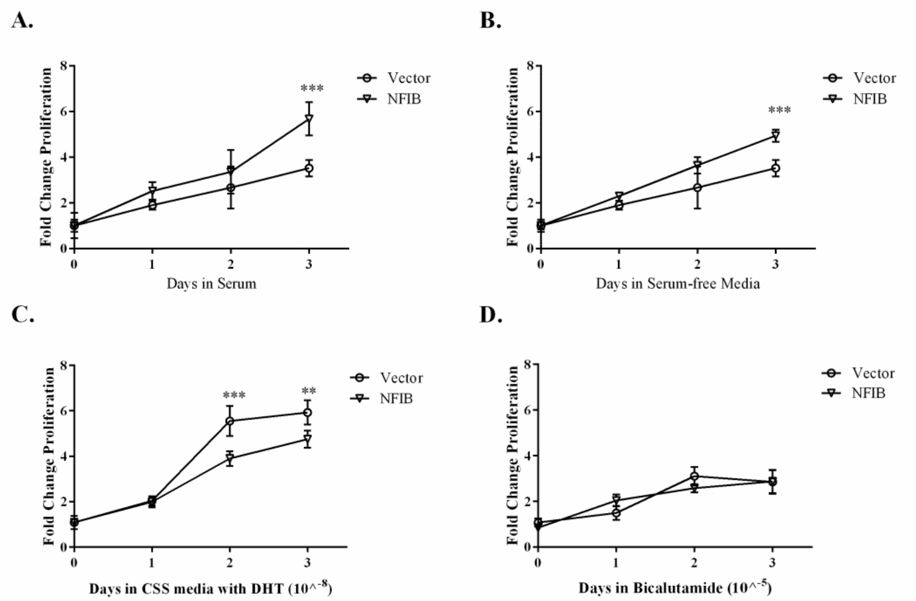

Figure 6: NFIB supports increased proliferation of 22RV1 cells. A. Proliferation assays comparing NFIB and vector control cell proliferation in serum (**A**), serum-free (**B**), DHT-supplemented (**C**), and bicalutamide (anti-androgen)-supplemented media (**D**). ** P< 0.01, *** P < 0.001

END OF UNPUBLISHED DATA

Specific Aim 2
Outside of the scope of the reporting period.

- **What opportunities for training and professional development has the project provided?**
  Training and professional development has been described in Training Tasks above.

- **How were the results disseminated to communities of interest?**
  Some of the results have been reported in a manuscript (under revision for Endocrinology [4]). The large data set generated by the ChIP-Seq analysis has been deposited into NCBI so that other investigators may interrogate the data set.

- **What do you plan to do during the next reporting period to accomplish the goals?**
  This award is being relinquished due to the awarding of a NCI K99/R00.

4. **IMPACT:**

- **What was the impact on the development of the principal discipline(s) of the project?**
  This project will have an impact on PCa research by further characterizing the role of NFIB in controlling AR expression and regulating NED. It also has the potential to define a novel biomarker for aggressive PCa.

- **What was the impact on other disciplines?**
  Nothing to report.

- **What was the impact on technology transfer?**
  Nothing to report.

- **What was the impact on society beyond science and technology?**
  Nothing to report.

5. **CHANGES/PROBLEMS:**

- **Changes in approach and reasons for change**
  Generation and characterization of cell lines took longer than anticipated and preliminary experiments were performed using transient transfections of previously published siRNA constructs targeting NFIB.

- **Actual or anticipated problems or delays and actions or plans to resolve them**
  Nothing to report.

- **Changes that had a significant impact on expenditures**
  Nothing to report.

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
  Nothing to report.

6. **PRODUCTS:**

- **Publications, conference papers, and presentations**
  A manuscript including some of the preliminary studies using transient transfections is under review at *Endocrinology* [4].

- **Journal publications.**
  Manuscript submitted and undergoing reversion[4].

- **Books or other non-periodical, one-time publications.**
  Nothing to report.

- **Other publications, conference papers, and presentations.**
  Poster presentation at the annual meeting of the SBUR[5].

- **Website(s) or other Internet site(s)**
  Nothing to report.

- **Technologies or techniques**
  Nothing to report

- **Inventions, patent applications, and/or licenses**
  Nothing to report

- **Other Products**
  Nothing to report.

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

- **What individuals have worked on the project?**

Name:	Magdalena Grabowska
Project Role:	PI
Researcher Identifier (ORCID ID):	0000-0003-1550-4925
Nearest person month worked:	12
Contribution to Project:	Dr. Grabowska has performed all experiments reported.
Funding Support:	Nothing to report.

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Changes for PI:

Magdalena M. Grabowska: New support
1K99 CA197315-01 Grabowska (PI) 09/01/15 - 08/31/17
Pathway to Independence Award (K99/R00)
Nuclear Factor I/B Action in Castrate Resistant Prostate Cancer
This application explores how nuclear factor I/B (NFIB) interacts with androgen receptor and androgen receptor splice variants to drive lethal castrate resistant prostate cancer, as well as how NFIB supports cytokine secretions that promote castrate-resistance.
Role: Principal Investigator

Changes for senior key personnel:

Robert J. Matusik: Support changed from current to completed

5R01 DK055748-14 Matusik (PI) 04/06/09 - 03/31/14
NIH/NIDDK

Control of Prostate-Specific Gene Expression
Our hypothesis is that by identifying the transcription factors (TFs) that control prostate-specific gene expression, we are also identifying TF that play a critical role in prostate development.
Role: Principal Investigator

4R01 CA076142-14 Matusik (PI) 08/11/09 - 06/30/14
NIH/NCI (no cost extension)
Transgenic Mouse Models for Prostate Cancer
The overall goal of our grant has been to characterize how the NF-κB and Pten pathway co-operate in the progression of androgen dependent prostate cancer to castrate resistant prostate cancer. This work will use both cell culture and transgenic animal models to test the role of these pathways during tumor progression.
Role: Principal Investigator

5P20 DK097782-02 Hayward (PI) 09/30/12 - 07/31/14
NIH/NIDDK
Obesity, Inflammation and BPH
This planning center will develop preliminary data for subsequent proposals. The center is focused upon the integrating research in benign urologic disease into the broader clinical context. In particular links between the urologic research group the obesity institute and diabetes centers will be expanded. Research will focus on the role of inflammation subsequent to obesity regulated by various means in benign prostatic growth using in vivo and clinical population based models.
Role: Co-Investigator

5R01 DK067049-08 Hayward (PI) 08/01/10 - 05/31/14
NIH/NIDDK
Paracrine Regulation of BPH Pathogenesis
This grant examines the role of inflammation and subsequent modifications to paracrine signaling in establishing a proliferative microenvironment in the prostate leading to benign enlargement. Methods to alleviate the response to a pro-inflammatory environment are explored.
Role: Co-Investigator

5R01 DK087962-04 Fowke (PI) 07/01/10 - 06/30/14
NIH/NIDDK
Biomarkers of Obesity, Prostate Tissue Inflammation, and BPH Progression
This is a prospective epidemiologic study on the relationships between obesity biomarkers and inflammation in BPH. It also investigates the mechanisms linking obesity on prostate hyperplasia utilizing two mouse models of obesity. This multidisciplinary project will advance our understanding of obesity as a risk factor for BPH and lead to interventions to delay BPH progression by targeting obesity and inflammation pathways.
Role: Co-Investigator

5U54 CA163072-02 Moses (PI) 09/23/11 - 08/31/16
NIH/NCI
MMC, VICC & TSU: Partners in Eliminating Cancer Disparities
Molecular Mechanisms of SKP2 Targeting on Prostate Cancer Progression

We have previously shown *in vitro* and *in vivo* that the acute inactivation of Pten leads to the aberrant upregulation of p19Arf, p53, p21, and that S-phase kinase-associated protein 2 (Skp2) is an important downstream target of PI3K-AKT pathway. Activation of Skp2 promotes cancer progression and Skp2 deficiency significantly inhibits tumor development in mouse models. We hypothesize that an inhibition of Skp2 activity suppresses castration resistant prostate cancer (CRPC). We propose to test this hypothesis and to

study the molecular interaction of Skp2 with Pten-p53 pathway in cells and how Skp2 inactivation impacts prostate cancer progression in mouse models.
Role: Co-Investigator

Peter E. Clark: Support recently awarded

3P30 CA068485-19S1 Pietenpol (PI) 09/01/14 - 08/31/16
NIH/NCI
Cancer Center Support Grant Supplement
This supplement is a NCI directed tissue procurement program for next generation sequencing of advanced prostate and bladder cancer.
Role: Co-Investigator

Peter E. Clark: Support changed from current to completed

5P20 DK097782-02 Hayward (PI) 09/30/12 - 07/31/14
NIH/NIDDK
Obesity, Inflammation and BPH
This planning center will develop preliminary data for subsequent proposals. The center is focused upon the integrating research in benign urologic disease into the broader clinical context. In particular links between the urologic research group the obesity institute and diabetes centers will be expanded. Research will focus on the role of inflammation subsequent to obesity regulated by various means in benign prostatic growth using in vivo and clinical population based models.
Role: Co-Investigator

3P20 DK097782-02S1 Hayward (PI) 08/01/13 - 07/31/14
NIH/NIDDK
Obesity, Inflammation and BPH Administrative Supplement
The purpose of this request is to generate data relevant to the appearance and consequences of inflammation in human BPH. This will enable further validation of the mouse models whose development and characterization is supported by the parent grant. Aim I: Characterize and quantitate inflammatory cells (M1 vs. M2 macrophages, CD4+Foxp3+ vs. CD8+ T cells) in the prostates of mouse models of obesity and diabetes as well as in incidental and advanced human BPH samples. Aim II: Profile the abundance of inflammatory lipids (leukotrienes, prostaglandins and aldehydes) and protein carbonylation in the prostates of mouse models of obesity and diabetes and in human BPH samples.
Role: Co-Investigator

5R01 DK087962-04 Fowke (PI) 07/01/10 - 06/30/14
NIH/NIDDK
Biomarkers of Obesity, Prostate Tissue Inflammation, and BPH Progression
This proposal includes a prospective epidemiologic study investigating the relationships between biomarkers of obesity and inflammation on BPH progression. It also investigates the mechanisms linking obesity on prostate hyperplasia utilizing two mouse models of obesity. This multidisciplinary project will advance our understanding of obesity as a risk factor for BPH and will lead to interventions to delay BPH progression by targeting obesity and inflammation
Role: Co-Investigator

- **What other organizations were involved as partners?**
  Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

- **COLLABORATIVE AWARDS:**
  Not applicable.

- **QUAD CHARTS:**
  Not applicable.

9. **APPENDICES:**

Appendix 1: References

1 Marchiani, S. *et al.* Androgen-responsive and -unresponsive prostate cancer cell lines respond differently to stimuli inducing neuroendocrine differentiation. *Int. J. Androl.* **33**, 784-793, doi:10.1111/j.1365-2605.2009.01030.x (2010).

2 Jin, R., Yamashita, H., Wang, J. & Matusik1, R. J. NF-kappa B signaling induces androgen receptor variants expression and restores responsiveness of castrate resistant prostate cancer cells to anti-androgen treatment., Submitted to Cancer Research (2013).

3 Grabowska, M. M. *et al.* NFI Transcription Factors Interact with FOXA1 to Regulate Prostate-Specific Gene Expression. *Mol. Endocrinol.* **28**, 949-964, doi:10.1210/me.2013-1213 (2014).

4 Grabowska, M. M. *et al.* NFIB loss supports prostatic hyperplasia and intermediate cell expansion. *Under revision for Endocrinology* (2015).

5 Grabowska, M. M. *et al.* in *Society of Basic Urologic Research* (Dallas, TX, 2014).

RRP - Case Manager Form

De-identified Study ID:_______

Race: ____White ____Black ____Asian ____Hispanic ____Indian (American) ____Other ____DNA

Pre-OP Info

PSA:_____ **Men's Wellness Class:**____Y____N____DNA

Salvage Case after Radiation: ____Y ____N

Hormones: Lupron/Casodex: ____Y ____N

5-ARI: Avodart/Proscar/Propecia: ____Y ____N

Classified as castrate resistant? _____ **Y** ____**N**

T Clinical Stage 2010:____TX____T0____T1a____T1b____T1c____T2a____T2b____T2c____T3a____T3b____T4 ____DNA

N Clinical Stage 2010: ____NX ____N0 ____N1

M Clinical Stage 2010: ____MX ____M0 ____M1 ____M1a ____M1b ____M1c

Metastatic locations: _______ ___________________________

Group: ____I ____IIA ____IIB ____III ____IV

Gleason Primary @BX: ___________

Gleason Secondary @BX: ___________

Total Gleason Score: ___________

SHIM: ____Y ____N, _______(1-25)

AUA SI: ____Y ____N, _______ (0-35)

OP Info

Pre-Op PCV:________ **Post-Op PCV:**________

BMI: _______, ___DNA

Post-OP Info

RRP - Case Manager Form - PATHOLOGY

Tumor % : _________________

Prostate Volume: __________________

Post-Op Tumor Grade: Gleason Primary: ___________

Gleason Secondary: ___________

Gleason Tertiary: ____________

Gleason Total: ___________

Margin Status: ____Positive ____Negative

Apex ____Y ____N

Base ____Y ____N

Lateral ____Y ____N

Anterior ____Y ____N

Posterior ____Y ____N

Bladder Neck ____Y ____N

Other ____Y ____N

T Stage 2010: ____TX ____T0 ____T2a ____T2b ____T2c ____T3a ____T3b ____T4 ____DNA

AJCC stage 2010: ____I ____IIA ____IIB ____III ____IV ____DNA

Seminal Vesicle Invasion: ____Y ____N

Nodol Involvement

____Not Sampled

____Not Lymph Tissue

____Sampled as Group	Total # Postive: _________	Total # Sampled: __________
Right Side	Total Right # Positive: _________	Total Right # Sampled: _________
Left Side	Total Left # Positive: _________	Total Left # Sampled: _________

Distant Metastasis: ____PCS(pre-clinical stage) ____M1 ____M1a ____M1b ____M1c ____DNA

Bladder Neck Invasion: ____Microscopic ____Gross ____None ____DNA

Extracapsular Extension: ____Y ____N ____DNA

NFIB DATA COLLECTION SHEET: Castrate Resistant PCa

Bone Resection or Channel TURP Case Manager Form

De-identified Study ID:_______

Race: ____White ____Black ____Asian ____Hispanic ____Indian (American) ____Other ____DNA

Pre-OP Info

PSA:_____ **Men's Wellness Class:**____Y____N____DNA

Salvage Case after Radiation: ____Y ____N
Hormones: Lupron/Casodex: ____Y ____N
5-ARI: Avodart/Proscar/Propecia: ____Y ____N
Additional Therapies Prior to Recurrence: ______________
____

At Time of Initial Prostate Cancer Diagnosis (if available)

T Clinical Stage 2010:____TX____T0____T1a____T1b____T1c____T2a____T2b____T2c____T3a____T3b____T4 ____DNA
N Clinical Stage 2010: ____NX ____N0 ____N1
M Clinical Stage 2010: ____MX ____M0 ____M1 ____M1a ____M1b ____M1c
Group: ____I ____IIA ____IIB ____III ____IV
Gleason Primary @BX: ___________
Gleason Secondary @BX: ___________
Total Gleason Score: ___________

OP Info

Procedure: ____Bone Resection ____Channel TURP

De-identified outcomes data

Record ID (auto-numbered and de-identified) ______________________

Race ______________________

Age (at prostatectomy) ______________________

Body Mass Index (BMI) ______________________

PSA (at diagnosis) ______________________

Salvage case after radiation? ______________________

Did patient receive neoadjuvant androgen deprivation therapy (ADT)?
- ◯ Yes
- ◯ No

Type of neoadjuvant therapy ______________________

Is patient considered castrate resistant at time of prostatectomy? ______________________

T 2010 Clinical Stage
- ◯ TX
- ◯ T0
- ◯ T1a
- ◯ T1b
- ◯ T1c
- ◯ T2a
- ◯ T2b
- ◯ T2c
- ◯ T3a
- ◯ T3b
- ◯ T4

N 2010 Clinical Stage
- ◯ NX
- ◯ N0
- ◯ N1

M 2010 Clinical Stage
- ◯ MX
- ◯ M0
- ◯ M1
- ◯ M1a
- ◯ M1b
- ◯ M1c

Location of metastatic deposits ______________________

Gleason primary @ BX ______________________

Gleason secondary @ BX ______________________

Total Gleason ______________________

Preop PCV ______________________

Post-op PCV ______________________

Pathology

Tumor Percent ______________________

Prostate volume ______________________

Post-op tumor grade: Gleason primary ______________________

Post-op tumor grade: Gleason secondary ______________________

Post-op tumor grade: Gleason tertiary ______________________

Margin Status
- ◯ positive
- ◯ negative

Margin Status: Apex
- ◯ Yes
- ◯ No

Margin Status: Base
- ◯ Yes
- ◯ No

Margin Status: Lateral
- ◯ Yes
- ◯ No

Margin Status: Anterior
- ◯ Yes
- ◯ No

Margin Status: Posterior
- ◯ Yes
- ◯ No

Margin Status: Bladder neck
- ◯ Yes
- ◯ No

Margin Status: Other
- ◯ Yes
- ◯ No

Pathology T 2010 Clinical Stage
- ◯ TX
- ◯ T0
- ◯ T1a
- ◯ T1b
- ◯ T1c
- ◯ T2a
- ◯ T2b
- ◯ T2c
- ◯ T3a
- ◯ T3b
- ◯ T4

AJCC stage 2010
- ◯ I
- ◯ IIA
- ◯ IIB
- ◯ III
- ◯ IV

Seminal vesicle invasion
- ◯ Yes
- ◯ No

Nodal involvement
- ◯ Not Sampled
- ◯ Not Lymph Tissue
- ◯ Sampled as Group

Nodal involvement: Sampled, right side ______________________

Nodal involvement: Postive, right side ______________________

Nodal involvement: Sampled, left side ______________________

Nodal involvement: Postive, left side ______________________

Total positive nodes ______________________

Total nodes sampled ______________________

Distant Metastasis
- ◯ MX
- ◯ PCS
- ◯ M1
- ◯ M1a
- ◯ M1b
- ◯ M1c
- ◯ DNA

Bladder neck invasion
- ◯ Microscopic
- ◯ Gross
- ◯ None
- ◯ DNA

Extracapsular invasion
- ◯ Yes
- ◯ No
- ◯ DNA

Recurrence

Did patient recur?
- ◯ Yes
- ◯ No

Time to biochemical recurrence (months) ______________________

Therapies subsequent to biochemical failure ______________________

Castrate Resistant Prostate Cancer

Did patient undergo bone resection or TURP to relieve compression or obstruction following failure of ADT?
- ◯ Yes
- ◯ No

Did patient undergo prostatectomy at VUMC/Has clinical data been previously entered?
- ◯ Yes
- ◯ No

Race ______________________

Age (at prostatectomy) ______________________

PSA (at diagnosis) ______________________

Salvage case after radiation?
- ◯ Yes
- ◯ No

Androgen deprivation therapy?
- ◯ Yes
- ◯ No

Therapy after failure of ADT ______________________

At primary diagnosis: clinical stage

- ◯ TX
- ◯ T0
- ◯ T1a
- ◯ T1b
- ◯ T1c
- ◯ T2a
- ◯ T2b
- ◯ T2c
- ◯ T3a
- ◯ T3b
- ◯ T4
- ◯ DNA

At primary diagnosis: N 2010 Clinical Stage

- ◯ NX
- ◯ N0
- ◯ N1

At primary diagnosis: M 2010 Clinical Stage

- ◯ MX
- ◯ M0
- ◯ M1
- ◯ M1a
- ◯ M1b
- ◯ M1c

At primary diagnosis: Group

- ◯ I
- ◯ IIA
- ◯ IIB
- ◯ III
- ◯ IV

At primary diagnosis: Gleason primary ______________________________

At primary diagnosis: Gleason secondary ______________________________

At primary diagnosis: Gleason sum ______________________________

Surgical procedure

- ◯ Channel TURP
- ◯ Bone Resection

Histological analysis

NFIB staining: tumor nuclear intensity ______________________________
(0 negative, 1 weak, 2 moderate, 3 strong)

NFIB staining: tumor nuclear distribution ______________________________
(0 negative, 1 < 33%, 2 33-66%, and 3 >66%)

NFIB staining: tumor sum ______________________________

NFIB staining: normal adjacent nuclear intensity ______________________________
(0 negative, 1 weak, 2 moderate, 3 strong)

NFIB staining: normal adjacent nuclear distribution ______________________________
(0 negative, 1 < 33%, 2 33-66%, and 3 >66%)

NFIB staining: NAT sum ______________________________

NFIB staining: CRPC nuclear intensity ______________________________
(0 negative, 1 weak, 2 moderate, 3 strong)

NFIB staining: CRPC nuclear distribution ______________________________
(0 negative, 1 < 33%, 2 33-66%, and 3 >66%)

NFIB staining of CRPC sample (sum) ______________________________

Androgen receptor status ______________________

Neuroendocrine differentiation status ______________________
(SYN, CHGRA, or FOXA2 + anywhere)

Is this prostate cancer classified as neuroendocrine PCa or small cell PCa?
- ◯ Yes
- ◯ No

NFIB staining: NEPCa nuclear intensity ______________________
(0 negative, 1 weak, 2 moderate, 3 strong)

NFIB staining: NEPCa nuclear distribution ______________________
(0 negative, 1 < 33%, 2 33-66%, and 3 >66%)

NFIB status in NEPCa (sum) ______________________